STATEMENT

by Assoc. Prof. Radoslava Emilova Grozdanova, PhD
National Center of Infectious and Parasitic Diseases-NCIPD,
Department of Immunology

regarding a PhD thesis for the award of the scientific degree "Doctor"
Field of higher education: **4. Natural Sciences, Mathematics and Informatics**
Professional field: **4.3. Biological Sciences**

Doctoral program: Immunology
Form of training: regular
Doctoral student: Vancho Donev
Scientific unit: Department of Immunology, NCIPD, Sofia
PhD thesis: "Characterization of the potential immunoprophylactic and immunotherapeutic properties of human blood plasma"
Scientific supervisor: Assoc. Prof. Dr. Georgi Nikolov, MD

The materials presented to me under the procedure are in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB, as well as the Regulations on the Conditions and Procedures for Acquiring Scientific Degrees and Holding Academic Positions at the National Center.
I declare that I have no conflict of interest with the PhD candidate.
The opinion was prepared based on the dissertation, its abstract and publications related to the topic.

Vancho Donev's dissertation examines intravenous immunoglobulin (IVIG) as an immunotherapeutic and immunoprophylactic agent in infectious diseases and significant aspects of the effectiveness and applications of COVID-19 convalescent plasma (CCP). The topic is very relevant and significant in connection with the increasing use of purified blood proteins - albumin, immunoglobulins and blood clotting factors as biological products in medical practice.

The structure of dissertation is classical and includes all the mandatory elements in the appropriate proportion, it is written on 130 standard pages and is illustrated with 23 figures and 7 tables. 10 conclusions are formulated and 5 theoretical and 3 applied contributions are identified. The bibliography includes a total of 317 sources.

The first part of the literature review is a very informative and comprehensive historical overview of the methods for the production of human blood products and their application for the treatment of chronic, life-threatening conditions, such as primary immunodeficiency diseases and blood clotting disorders. The application of convalescent plasma (CP) for the treatment of numerous viral infectious diseases and in emerging epidemics is examined. The development of intravenous immunoglobulin products (IVIG) and their application in bacterial, fungal and viral infections is also discussed. The production of albumin and immunoglobulin solutions and Immunovenin-intact using original technologies in our country is described very comprehensively.

The presentation also addresses the mechanisms for regulation and modulation of the immune response by IVIG, which is directly related to their application in the prevention and replacement therapy of infectious diseases.

The historical sequence of the exposition, the physiological mechanisms of action and the emphasis on the need for further research regarding the therapeutic properties and standardization of blood products at the end of the literature review, allows the author to argue the purpose of the dissertation:"study of the main immune components of human blood plasma used for the production of the final IVIG product Immunovenin Intact 5% and characterization of the pathogen-specific antibodies contained, with a view to possible immunoprophylactic and immunotherapeutic application."

To achieve the set goal, six main tasks have been set, which are logically structured based on the literature review and the need for additional studies regarding the immunological characteristics of IVIG.

The selected methods are modern and have high sensitivity and specificity, thus contributing to the reliability of the results.

Three main research methods are used in the development:

- ✓ ELISA for detection of SARS-CoV-2-specific RBD-IgG and IgA, specific IgG against C. albicans, anti-Alpha-hemolysin (Hla) IgG from S. aureus and ANA screening.
- ✓ Multiplex microsphere method for determining the main classes and subclasses of immunoglobulins, cytokine profile and neutralizing antibodies against the different variants of SARS Cov-2.

- ✓ Immunoblot for ANA characterization in individual samples from the ANA screening.

All methods are very well and in detail described and the precise development of a home-made ELISA method for assessing anti-Alpha-hemolysin (Hla) from S. aureus makes a good impression.

The original results obtained from the study have been discussed in detail and competently, with a thorough analysis being conducted and 10 conclusions being formulated that logically follow the stated goals and objectives of the study.

For me, some of the most significant contributions of the dissertation are:

- ✓ The characterization of immunoglobulin subclasses shows that the average concentration of IgG subclasses in IVIG reproduces typical values for human plasma, while the remaining subclasses are in minimal concentrations, with variations in different batches being below 2%.
- ✓ Intravenous immunoglobulin has higher neutralizing activity compared to convalescent plasma (CCP), in which 44% of samples showed a lack of specific RBD-IgG and IgA SARS-CoV-2 antibodies.
- ✓ High concentrations of pro-inflammatory cytokines were not detected in plasma pools from intravenous immunoglobulin and SSR from the period of the COVID-19 pandemic.
- ✓ Development and validation of an ELISA method for the determination of IgG specific antibodies against the hemolysin toxin of S. aureus.
- ✓ The ANA screening of intravenous immunoglobulin and subsequent ANA profile revealed the presence of Ro-52 and AMA M2 in two and SS-A in one of the IVIG batches, which should be taken into account in serological tests performed after IVIG administration.

I accept the formulated 5 contributions of a theoretical nature and three of a scientifically applied nature and I believe that they objectively reflect the real results of the conducted studies.

Vancho Donev is the first author oftwo publications in journals with an impact factor and one publication in a journal indexed in Scopus, in which the results of the dissertation work are presented. He has also participated in two international and four national scientific forums on the topic.In terms of number and quality, the scientific works fully meet the requirements for the Doctoral Degree, according to the Regulations of the National Center for Scientific Research, Sofia.

In conclusion, Vancho Donev's doctoral dissertation meets the regulatory requirements and is the result of the doctoral student's original work. It addresses current issues related to the characterization and standardization of human blood products used in modern medicine. The specific tasks that ensure the achievement of the main goal of the dissertation work have been

successfully completed and the results obtained lead to the formulation of significant conclusions and contributions.

The dissertation shows that Vancho Donev possesses in-depth theoretical knowledge and professional skills in the scientific specialty, demonstrating qualities and skills for independently conducting scientific research.

Everything stated so far gives me reason to give a positive assessment of the research conducted and I propose to the members of the esteemed scientific jury to award Vancho Doneveducational andscientific degree "Doctor" in the scientific specialty "Immunology", Professional field: Biological Sciences.

Prepared the opinion: .....

18. 08. 2025

Assoc. Prof. Radoslava Emilova Grozdanova, PhD